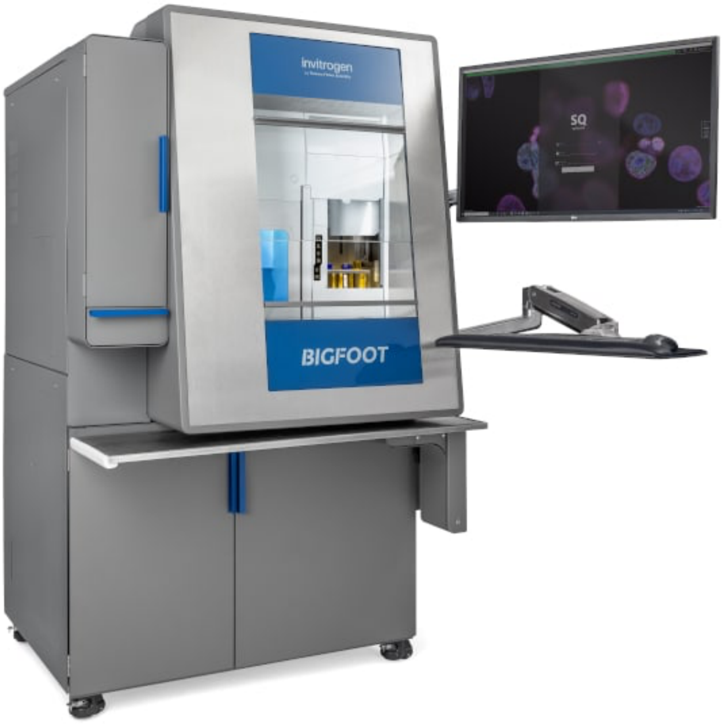
Bigfoot Standard Operating Procedures



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Starting up Bigfoot

Always keep an eye on the status bar in the top of the screen.

1. If the Bigfoot is off, turn on both the instrument and the PC on the right side of Bigfoot.

2. Sign in to Windows with credentials:

   User: Bigfoot

   Password: XXXX

3. Open *SQ Launcher* SQ.

4. If there are updates Update (4), please do not update, but tell the FACS Core staff that an update is pending.

5. Launch the software Launch.

6. Sign in to your account.

7. If Startup is available ☼ click this, if not then look at warnings.

8. Choose temperature for today's experiment and click *Yes*.

9. Open the *QC protocol* that is pinned to the top of the list.

10. Check in the upper right corner if the nozzle settings are correct for your experiment.

    If not, go to ⌄ Flex Controls

    → *Maintenance*

    → *Swap Tip* and follow the Wizard.

The replaced nozzle must be cleaned before stored by doing the following:

→ Place the nozzle in a 15 ml tube with 3 ml cleaning solution (2% Helmanex III).

→ Whirlmix the tube for 30 seconds with a 30-45° angle so the nozzle jumps up and down.

→ Place the nozzle in a new 15 ml tube with 3 ml DI-H_2O and repeat the whirlmixing.

→ Replace the DI-H_2O and repeat whirlmixing again, ensuring there is no residue from the cleaning solution.

→ Dry the nozzle with canned air.

→ Look at the nozzle under a microscope to check if it is clean.

→ If not, repeat the process above.

→ If it is not clean after the second time, sonicate the nozzle in a cleaning solution for 1 minute.

→ Ensure there is no residue from the cleaning solution.

→ Sonicate the nozzle in DI-H_2O for a few minutes.

→ Look at the nozzle under a microscope to check if it is clean.

→ If not, repeat the sonication process.

11 If you need aseptic sample lines for culturing sorted cells place a 15 ml tube with 70% ethanol in position 5 of the carousel and a 5 ml tube of DI-H_2O in position 6

→ Go to ⌄ Samples and click + Add Sample twice and change their positions to 5 and 6

→ Mark the position 5, go to ⌄ Control Panel and set *Flow Rate* to 250%, click ▷

→ Let it run for 20 minutes → Then click ⏩

→ Let it run for 10 minutes → Then click ⏹

12 If you did not execute step 11, then wait 10 minutes for the stream to stabilize before continuing with step 19.

Step 13, 14 and 15 can be performed while waiting the 10 minutes.

13 Go to ⌄ Flex Controls → *Maintenance*, open the *Nozzle Mover* panel

→ Turn on Pinhole Light

→ If *Image Match* is > 0.9, continue with step 18, if not, click in the X/Y box and use arrow keys to move nozzle until *Image Match* is > 0.9.

→ Turn off Pinhole Light

→ *Save Nozzle Position*

14 Go to Flex Controls → *Maintenance* → *Swap Beads*

→ Get an ampule of beads from the refrigerator and resuspend the beads thoroughly

→ Insert beads to the bead position

→ *Swap Beads*

15 If you want automatic Bead Alignment, skip this step.

Go to Flex Controls → Setup → *Bead Alignment* → *Settings* ⚙

→ Uncheck all options to the right

→ Check *Run Manual Bead Alignment*

→ Keep *Run MFI Voltage Optimization* checked, if you setting up a new spectral experiment.

16 Click ✓ in the upper right corner and Click Custom QC

Custom QC completes all the steps automatically until bead alignment, where it will pause after approximately 5 minutes and you must do the bead alignment manually. For manual adjustment of Bead Alignment, see next SOP “Manual Bead Alignment”.

The steps from Custom QC can be manually performed at Flex Controls → *Setup*

17 When Custom QC procedure is finished the Drop Delay report window will appear. Click Done.

18 Go to sidebar menu and click *Reports* 🗎

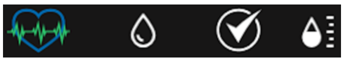
→ *QC – Bead Alignment Reports*

→ Confirm that today’s CV values are below 6%

→ Click *Drop Delay Reports*

→ Check values of latest Drop Delay. **Confidence value should be higher than 0.95**.

The instrument is ready when the icons in the top right of the screen looks like this:



Manual Bead Alignment

When doing manual bead alignment, you move the nozzle in the Y or X axis as seen in figure 1.

If the lasers do not hit the stream from the nozzle correctly, your measurements will not be adequate because your cells will not be centred in the laser beams.

This will be displayed in the plots as wide peaks or having several peaks. See figure 2.

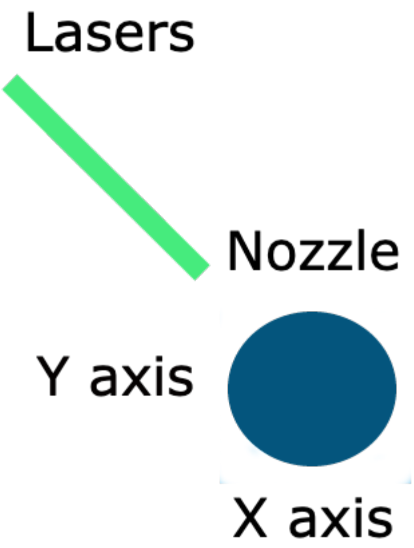
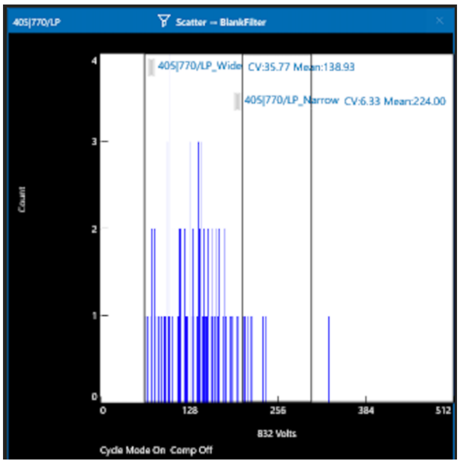


Figure 1: Aligning nozzle to lasers.



405|770/LP_Wide CV:35.77 Mean:138.93

405|770/LP_Narrow CV:6.33 Mean:224.00

Figure 2: Wide and uncentred peak.

The Bead Alignment wizard will adjust event rates and build plots and will pop up with a window to the left of the screen where you can adjust the position of the nozzle.

The goal of manual alignment is to get the peaks in the plots to be as narrow as possible. See figure 3.

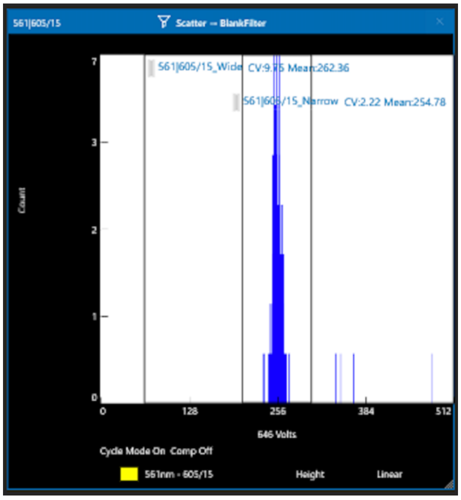
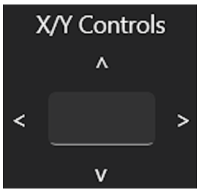


Figure 3: Nice and centered peak.

1 Click in the X/Y box 

→ Start by using ⇑ and ⇓ on the keyboard to move the nozzle in the Y axis.

→ Use ⇒ and ⇐ on the keyboard to move the nozzle in the X axis.

→ Peaks in the plots will move and change width.

If you cannot get a narrow peak in a plot, then it is preferable to have the peak in the right side of the plot.

2 Click *Adjust Voltages.*

3 Repeat step 1 and 2 until you are satisfied with every peak width.

4 Click *Accept Bead Alignment* and the software will collect 5.000 events.

Compensation: Creating a new protocol and acquiring compensation controls

1 Click on New Sort

→ Rename protocol

→ Add description and tags

→ Next →

→ Select No for spectral unmixing experiment No

2 Search for dyes in the top left of the screen and add them to your protocol

   a. If a dye is not available, check the dye in a spectra viewer and choose the optimal laser and filter.

3 Add prefixes, if any, to the channels.

4 Click →

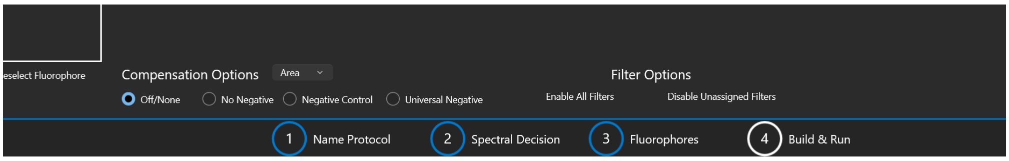
5 Choose compensation setup and controls in the bottom of the screen:

Off/None	No controls will be added. Use this for experiments with no compensation.
No Negative	No negative control will be added. Use this if all your compensation controls include a negative population (eg. eComp beads) and you do not have an unstained control.
Negative Control	**This is the recommended option in most cases.** Adds a negative control from which you will acquire your unstained cells to apply a correction for autofluorescence. All your single stain controls must have both a positive and a negative population. This option also opens up for compensating on heterogeneous samples. This is described in step 9.
Universal Negative	Adds a negative control and uses it as the negative population in all your compensation controls. Use this only for a homogeneous sample e.g., cell culture, and if you use cells only for single stain controls.

6 In the lower part of the screen:

a. Choose compensation option: *Height* or *Area*

b. Under filter options you can check *Enable all filters* if you want that or *Disable Unassigned Filters*


Compensation Options: Area
● Off/None ○ No Negative ○ Negative Control ○ Universal Negative

7 The software has already assigned positions to your compensation controls

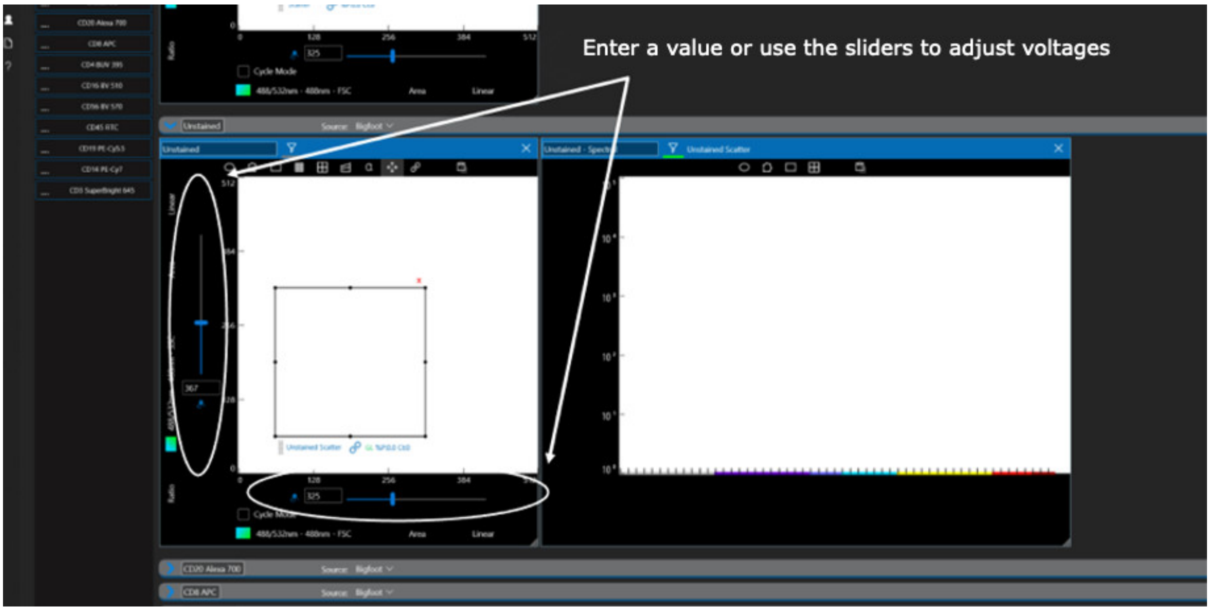
→ Place the controls in the sample loader

If you want to import FCS files from former compensation controls, skip step 9-12 for each control with an FCS file.

8 Click on a control

→ *Acquire* ▷

→ Adjust *Volts* of the axes by entering a value or using the slider



Enter a value or use the sliders to adjust voltages

→ Click ⟳ to refresh the plots

9 Adjust gates according to good practices in compensation.

To make different gates for individual cell types do the following:

→ Go to **Samples** and click on the single stain control for one of your cell types

→ Right click the gate in the FSC vs SSC plot

→ Click the + under **Link**

→ A new linked gate is created and can be named e.g., Lymphocytes

→ Click on it so the light blue vertical line to the left is present

→ In all other single stain controls for markers on lymphocytes, right click the gate in the FSC vs SSC plot and choose the Lymphocytes gate under link

→ When you change this gate in one control it will change in all others with the chosen gate

→ Repeat above procedure for other cell types

10 Record ○

11 If you are using cells as compensation controls clean thoroughly according to **Cleaning between samples** SOP. If you have been using beads, the instruments basic cleaning between samples is usually enough.

12 Repeat step 8 to 11 for all controls

13 Click Auto Comp

→ The wizard will include the data from step 8-11 by default

14 If you want to import FCS files to your compensation, for each control click 🗋

→ Find the relevant FCS file

→ *Open*

→ →

15 Review gates in your compensation plots

→ →

→ *Ok*

16 If you are satisfied with your compensation, click ✓

Spectral Flow Cytometry: Creating a protocol and acquiring unmixing controls

1 Click on New Sort

→ Rename protocol

→ Add description if wanted

→ Type tags and click add

→ Next →

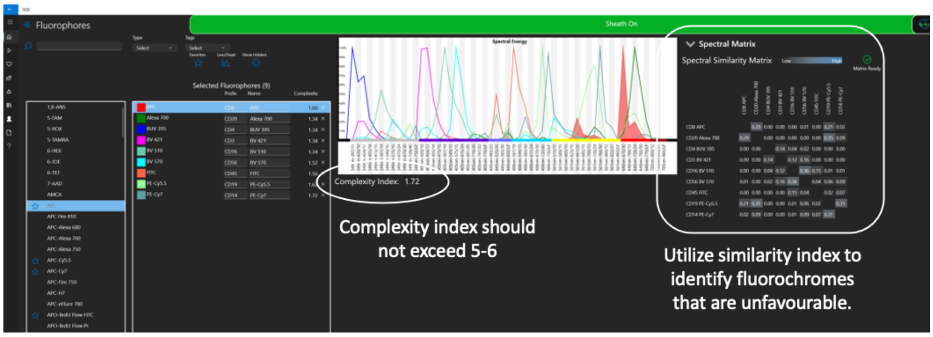
→ Click *Yes* to the popup *Spectral Unmix Experiment?*

→ Yes

2 Search for dyes in the top left of the screen and add them to your protocol.

a. If a dye is not available, find one with similar spectral emission and add it. Please tell the FACS Core staff if this occurs and we will contact Thermo Fisher to have them add the fluorophore to the library.

b. Pay attention to the complexity index and the spectral similarity index. The complexity index should not exceed 5-6, but if it does, look in the similarity index to identify similar fluorophores. Reconsider highly similar fluorochromes or make sure these fluorophores are on mutually exclusive markers.



Selected Fluorophores (9)

	Prefix	Name	Complexity
APC	CD8	APC	1.00
Alexa 700	CD20	Alexa 700	1.34
BUV 395	CD4	BUV 395	1.34
BV 421	CD3	BV 421	1.34
BV 510	CD16	BV 510	1.34
BV 570	CD56	BV 570	1.52
FITC	CD45	FITC	1.56
PE-Cy5.5	CD19	PE-Cy5.5	1.6
PE-Cy7	CD14	PE-Cy7	1.72

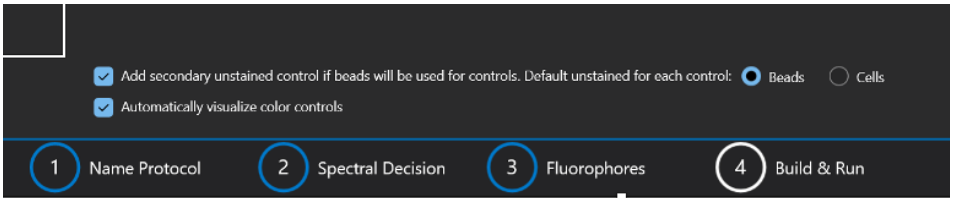
Complexity Index: 1.72

Complexity index should not exceed 5-6

Utilize similarity index to identify fluorochromes that are unfavourable.

3 Add prefixes, if any, to the selected fluorophores.

4 If you are using beads for control, check the *Add secondary unstained control* box in the bottom of the window and select whether beads or cells will be your default (you will be able to change this for each sample later).



☑ Add secondary unstained control if beads will be used for controls. Default unstained for each control: ● Beads ○ Cells

☑ Automatically visualize color controls

5 Check the box *Automatically visualize color controls*.

6 Click →

(Go to step 21 if you want to import settings and/or controls from a previous protocol)

7 The software automatically generates the samples required to acquire negative and single stain controls.

If you, in the previous step checked beads as controls, a sample with unstained cells as well as one for unstained beads will automatically be added.

8 Create a sample and the following plots:

→ FSC-A vs. SSC-A plot

→ FSC-A vs. FSC-H plot

→ SSC-A vs. SSC-H plot

→ Spectrum plot

9 Acquire ▷ a small aliquot of your unstained cell sample and adjust forward and side-scatter voltages to find your cells: Do not record yet.

→ Activate the FSC-A vs. SSC-A plot by clicking on it

→ Adjust *Volts* of the parameter by entering a value or using the slider

→ Click 🔄 to refresh the plots.

→ Create a gate around your cells in the FSC-A vs. SSC-A plot

→ Show the cell gate in the FSC-A vs. FSC-H plot and create a FSC singlet gate

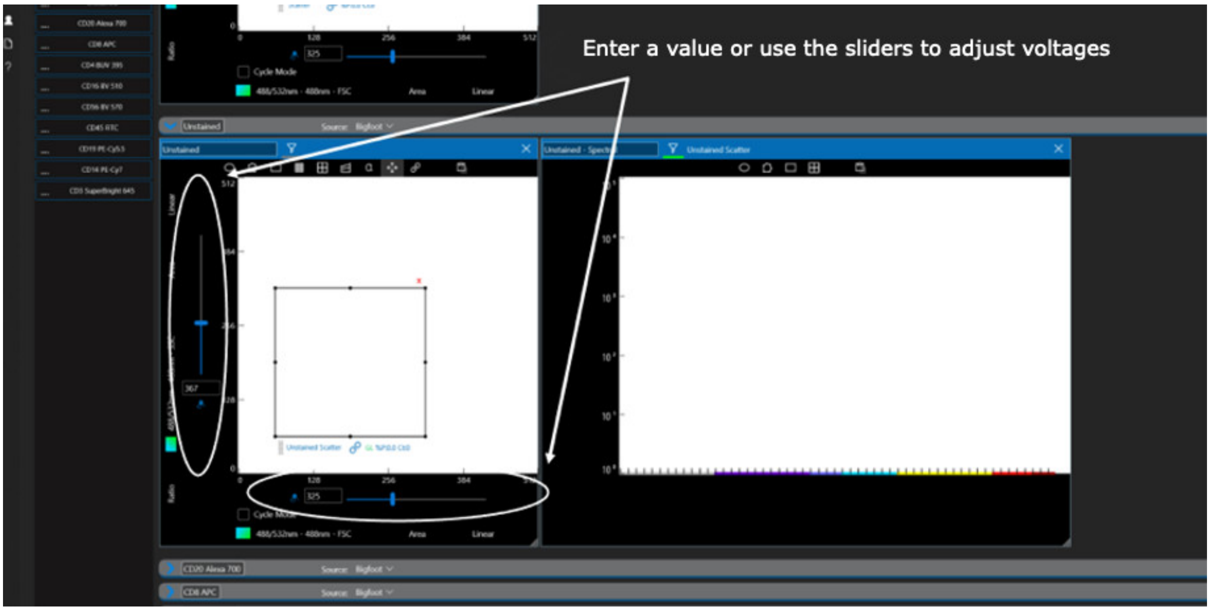
→ Show the FSC singlet gate in the SSC-A vs. SSC-H and create a SSC singlet gate

→ Show the SSC singlet gate in the spectrum plot

10 Acquire ▷ a small aliquot of your all stain sample without recording

→ Look in the spectrum plot and check if all events are on scale

→ If any events are off scale go to **Flex Controls** and *Detection* and reduce voltage either on a single detector, or using the Sync mode and lower the voltage of all detectors on a single laser simultaneously*, or see picture to change voltages in plots.



Enter a value or use the sliders to adjust voltages

→ Click 🔄 to refresh the plots and repeat until all events are on scale

11 If beads are used for unmixing, take 10 µl from all of your single stain controls and mix in a tube. If the volume is less than 100 µl add some PBS.

→ Acquire ▷ the mix without recording

→ Look in the spectrum plot and check if all events are on scale

→ If any events are off scale, evaluate which fluorophores could be the cause and make a new control with a lower antibody concentration than before and repeat.

12 Place the controls in the proper positions in the sample loader.

  a. If you are using cell and bead controls, there will be a little B or C next to each sample. Make sure to assign proper negative control to each sample (click once on the letter to change).
  b. If you are using heavy beads that sediment quickly, (such as Ultracomp beads from Thermo Fisher) you can increase the agitation speed of the samples to “Ultra” (tornado icon On).

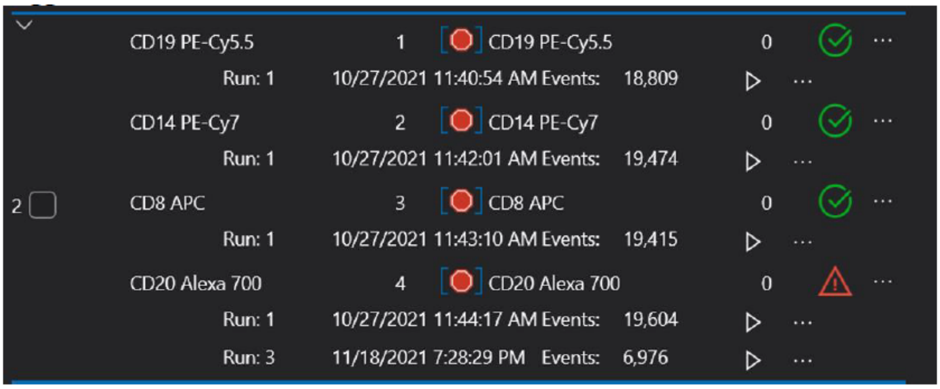
13 Right click inside a gate to remove *Link gate* so the FSC-SSC gate can be moved between cells and beads.

14 Acquire each single stained control. Record a proper number of events for each sample (for beads 5.000 events in total. For cells at least 200 positive events and minimum 20.000 events in total): Mark the sample(s) in your sample list and click *Record* ○. The carousel can take up to 6 samples at a time, so repeat until all single stain samples have been recorded.

  a. If you are using cells as unmixing controls clean thoroughly according to **Cleaning between samples** SOP. If you have been using beads, the instruments basic cleaning between samples is usually enough.
  b. Beads may sediment even though you have mixing set on ultra. When one sample is acquiring, you can take out the next sample in line and vortex it.

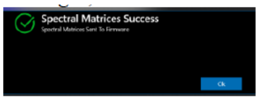
15 A green checkbox appears next to the sample when it has been recorded and the data has been unmixed. If there are errors during the unmixing, they will be marked by orange or red symbols. Hover the mouse over the symbols to view errors and get suggestions to resolve issues. Red errors must be corrected to be able to continue: the

software uses the Visual control plot. You can change the gate if needed. If the error is still not resolved, you may have to make a new control sample.



	CD19 PE-Cy5.5		1	CD19 PE-Cy5.5	0	✓
		Run: 1	10/27/2021 11:40:54 AM	Events:	18,809	▷
	CD14 PE-Cy7		2	CD14 PE-Cy7	0	✓
		Run: 1	10/27/2021 11:42:01 AM	Events:	19,474	▷
2 ☐	CD8 APC		3	CD8 APC	0	✓
		Run: 1	10/27/2021 11:43:10 AM	Events:	19,415	▷
	CD20 Alexa 700		4	CD20 Alexa 700	0	⚠
		Run: 1	10/27/2021 11:44:17 AM	Events:	19,604	▷
		Run: 3	11/18/2021 7:28:29 PM	Events:	6,976	▷

16 After all samples are acquired (and possible errors are resolved) a *Spectral Matrices Success* message appears. Click *OK*

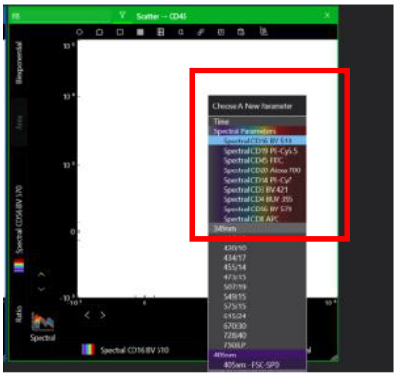


Spectral Matrices Success

Spectral Matrices Sent To Firmware

17 Unmixed FCS files will be generated automatically.

18 Add extra 2D plots. Visualize the unmixed single stained controls (these will be at the top of the list, see the red box below). Check if you are satisfied with the unmixing. If not, look at the Visual control plot for each single stained sample and change the gate, if needed. If you change anything, you must recalculate the unmixing.



19 Acquire a small aliquot of the all-stained sample

→ *Record* ○

→ *Stop* ■

20 Create your gating strategy with the unmixed spectral parameters on your axis.

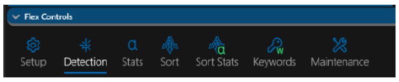
21 It is possible to import detector values from a previously run protocol (Note: Default detector values are set from a successful QC). To import values from a previous protocol, go to **Flex Controls**

→ *Detection*

→ *Import*

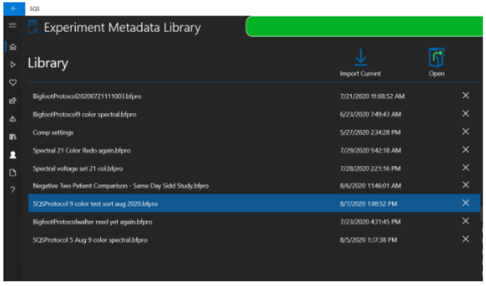
→ Select desired protocol from list in the library

→ *Apply*



Click **Import**.

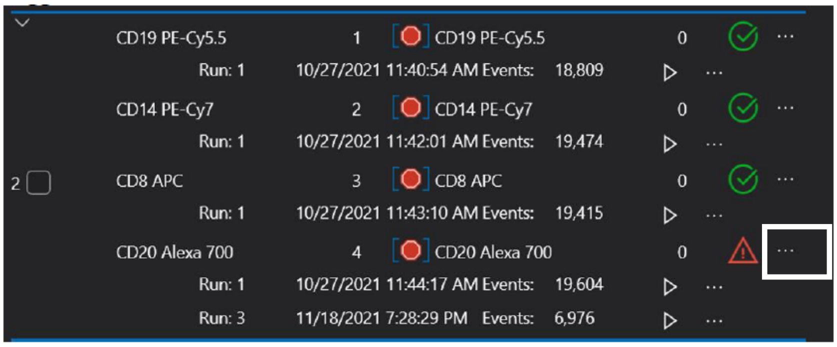
Select the protocol from the list in the **Library**.



BigfootProtocol20200721111003.bfpro	7/21/2020 11:08:52 AM
BigfootProtocol9 color spectral.bfpro	6/23/2020 7:49:43 AM
Comp settings	5/27/2020 2:34:28 PM
Spectral 21 Color Redo again.bfpro	7/29/2020 9:42:18 AM
Spectral voltage set 21 col.bfpro	7/28/2020 2:21:16 PM
Negative Two Patient Comparison - Same Day Sidd Study.bfpro	8/6/2020 11:46:01 AM
SQSProtocol 9 color test sort aug 2020.bfpro	8/7/2020 1:08:52 PM
BigfootProtocolwalter reed yet again.bfpro	7/23/2020 4:31:45 PM
SQSProtocol 5 Aug 9 color spectral.bfpro	8/5/2020 1:37:38 PM

Click the **Apply** button. This will apply voltages from the selected protocol to the current experiment.

22 To import an FCS file from a previous experiment, e.g. if you want to re-use your single stained control from a previous experiment, you must first import detector values and then the FCS file: Click on the three dots to the right of a sample (see the white box below) and choose import FCS file.



CD19 PE-Cy5.5 1 CD19 PE-Cy5.5 0 ✓ ···
Run: 1 10/27/2021 11:40:54 AM Events: 18,809 ▷ ···

CD14 PE-Cy7 2 CD14 PE-Cy7 0 ✓ ···
Run: 1 10/27/2021 11:42:01 AM Events: 19,474 ▷ ···

2 ☐ CD8 APC 3 CD8 APC 0 ✓ ···
Run: 1 10/27/2021 11:43:10 AM Events: 19,415 ▷ ···

CD20 Alexa 700 4 CD20 Alexa 700 0 ⚠ ···
Run: 1 10/27/2021 11:44:17 AM Events: 19,604 ▷ ···
Run: 3 11/18/2021 7:28:29 PM Events: 6,976 ▷ ···

Sorting on Bigfoot

1 Right click the gate you want to assign as sort gate

→ Assign sort gate ⇡Sort ● On

→ Repeat for all sort gates.

2 Click ⇡ in the top of ⌄ Control Panel (this pauses any ongoing acquisition of a sample)

3 Decide your Sort Logic:

Change Media Type	Sort Media Options:	Sort Limit:	Sort Flip Polarity Volume:
Choose from tubes or plates.	Tube size or plate types.	Amount of cells you want sorted.	Click ? for explanation.

4 **Repeat this step for each sort fraction:**

→ Select a position

→ Select *Gate*

→ Check ● Details

→ Set *Output Starting Volume* of the collection tube

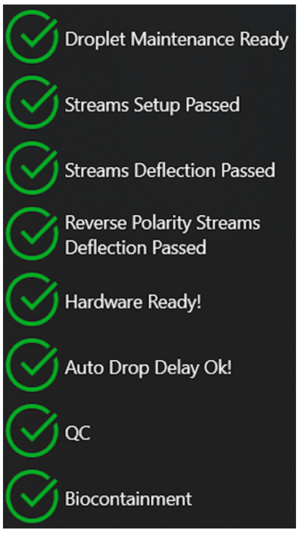
→ Choose *Priority* of the fraction

→ Select *Mode*

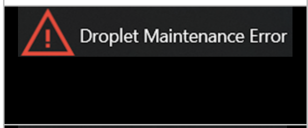
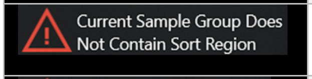
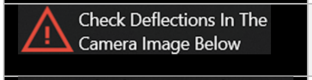
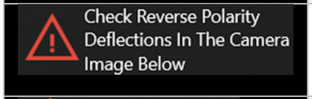
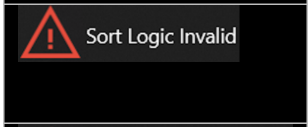
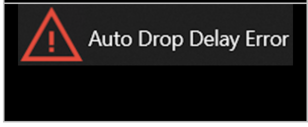
5 Go to ⌄ Validation in the bottom of the screen and check the streams deflection by clicking Check Streams Deflection and Check Reverse Polarity Streams Deflection, both followed by Confirm when if you are satisfied with the streams.

Assure that the streams hit the ▼ in the stream window.

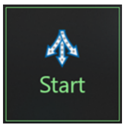
6 When you have completed the steps above and the SOP **Starting up the Bigfoot**, the Sort Pre-Check Status should look like this:



✓ Droplet Maintenance Ready
✓ Streams Setup Passed
✓ Streams Deflection Passed
✓ Reverse Polarity Streams Deflection Passed
✓ Hardware Ready!
✓ Auto Drop Delay Ok!
✓ QC
✓ Biocontainment

Possible errors	Reasons and solutions
 Droplet Maintenance Error	Startup SOP has not been completed or sheath is paused.
 Current Sample Group Does Not Contain Sort Region	See step 1 in this SOP.
 Check Deflections In The Camera Image Below	See step 5 in this SOP.
 Check Reverse Polarity Deflections In The Camera Image Below	See step 5 in this SOP.
 Sort Logic Invalid	Gate may not have been selected at step 4 in this SOP.
 Auto Drop Delay Error	Startup SOP has not been completed or sheath is paused.

7 To save an FCS file based on number of all events, enter a value at "*Save FCS file*".
If you need Index Sorting for single cell sorting, then the value must be 0, but keep in mind that this means that the Bigfoot keeps recording until your plate is full. If your target population is rare this may mean large FCS files that can slow down the system. Consider if you need Index Sorting in this situation.

8 Start the sort 

9 When the sort is finished, click "*Share*" to the sort report that pops up

→ Choose any items you want to include in the sort report

→ Click *Print* and *Print* in the popup

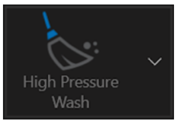
→ Choose a location and click *Save*

Cleaning between samples

1 Place a 15 ml tube with a cleaning solution (ammonium free g*lass cleaner) in position 1

→ Go to and *Maintenance* 🛠

Flex Controls

→ Expand the High Pressure Wash by clicking the arrow 

→ Check *Pulsing* and *Custom Wash Time* and change the wash to 30-60 seconds depending on how sticky your cells are

→ Start *High Pressure Wash*

2 Check *Cycle Mode* and set it to 10 seconds and Acquire DI-H_2O at 250% Flow Rate until Ct in your primary cell gate is acceptably low (we recommend maximum 2 events)

→ Unload 🟥

→ Change flow rate back to your original value

→ Uncheck *Cycle Mode*

Tips:

In case of extremely sticky cells it may be required to increase the time for High Pressure Wash until the Ct in your primary cell gate is low.

Keeping an eye on your threshold window can give an indication whether or not your sample line is clean.

Cleaning in between users

1 Look in the Bigfoot calendar to check if someone is going to use the cell sorter after you and call the person. If you are the last user, skip this session and go to *"Shutting down Bigfoot"* on page 24.

2 If you have been analyzing or sorting bacteria the Bigfoot must be shut down with an extensive procedure before another user can operate it. Follow the SOP "*Shutting down Bigfoot*" and finish by calling the next user when the whole shutdown procedure is performed and remember to tell them that the Bigfoot has been shut down.

3 Place a 15 ml tube with a cleaning solution in position 1

→ Go to Flex Controls and *Maintenance*

→ Expand the High Pressure Wash by clicking the arrow High Pressure Wash

→ Check *Pulsing* and *Custom Wash Time* and change it to 180 seconds

→ Start *High Pressure Wash*

4 If you have been using DNA dyes install a 15 ml tube with 15% bleach and acquire for 5 minutes at 250% Flow Rate, if not continue.

5 Acquire DI-H_2O at 250% Flow Rate while looking at the plots from your experiment and acquire until the Ct (count) in your FSC-SSC plot is at maximum 2 with a cycle of 10 seconds.

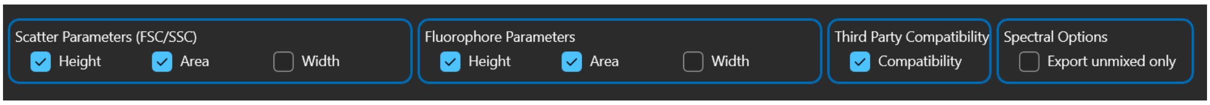
6 Go to Flex Controls

→ *Maintenance*

→ *Pause Sheath*

7 Go to *Export* in the sidebar menu

→ Select the parameters you would like to export from the top of the screen



Scatter Parameters (FSC/SSC)
☑ Height ☑ Area ☐ Width

Fluorophore Parameters
☑ Height ☑ Area ☐ Width

Third Party Compatibility
☑ Compatibility

Spectral Options
☐ Export unmixed only

→ *Select all* Select all from your current experiment

→ D:\PropelExport and select a folder on the server

→ *Select folder*

→ *Start pending exports*

8 Transfer data and reports to yourself.

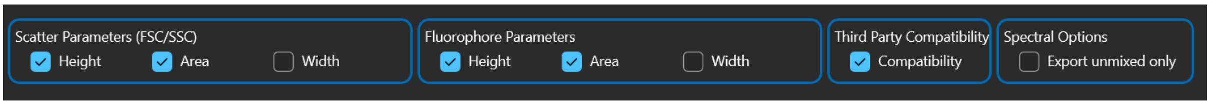
9 Logout of the software.

Shutting down Bigfoot

1 Look in the Bigfoot calendar to confirm that no one is using the instrument after you.

2 Go to *Export* in the sidebar menu

→ Select the parameters you would like to export from the top of the screen



Scatter Parameters (FSC/SSC) ☑ Height ☑ Area ☐ Width

Fluorophore Parameters ☑ Height ☑ Area ☐ Width

Third Party Compatibility ☑ Compatibility

Spectral Options ☐ Export unmixed only

→ *Select all* Select all from your current experiment

→ D:\PropelExport and select a folder on the server

→ *Select folder*

→ *Start pending exports*

3 Transfer data and reports to yourself.

4 **If you have been sorting or analyzing bacteria skip to step 10.**

5 Place a 15 ml tube with a cleaning solution in position 1

→ Go to Flex Controls and *Maintenance*

→ Expand the High Pressure Wash by clicking the arrow High Pressure Wash

→ Check *Pulsing* and *Custom Wash Time* and change it to 180 seconds

→ Start *High Pressure Wash*

6 If you have been using DNA dyes install a 15 ml tube with 15% bleach and acquire for 5 minutes at 250% Flow Rate, if not continue.

7 Acquire DI-H_2O at 250% Flow Rate while looking at the plots from your experiment until the Ct (count) in your FSC-SSC plot is at maximum 2 with a cycle of 10 seconds.

Skip step 8.

8 For cleaning after bacteria set flow rate to 250% and do the following:

a. Acquire 10% Bleach for 10 minutes.
b. Acquire Glass cleaner for 5 minutes.
c. Acquire 70% Ethanol for 10 minutes.
d. Acquire 2% Hellmanex for 5 minutes.
e. Acquire DI-H_2O for 5 minutes.
f. Dispose all of the above tubes as waste.

9 Remove all tubes from the carousel.

10 Go to Flex Controls

→ *Maintenance*

→ *Swap Beads*

→ Place the ampule with QC beads back in the refrigerator.

→ *Swap Beads*

11 Go to Flex Controls

→ *Maintenance* 🛠

→ Click *Swap Bulk Fluid Tanks* Swap Bulk Fluid Tanks (Top Active)

12 Go to *Home* ⌂, click *Shutdown* ☾ and choose:

a. **Regular cells:** *Quick Sample Line Decon and Rinse* (it takes approximately 20 minutes).
b. **Bacteria:** *Full Sample Path Decon* (it takes approximately 1 hour).

Place a 15 ml tube with 15% bleach in position 1 as requested by the software and click *Ok.*

Step 13, 14 & 15 can be performed while shutting down.

13 Put on gloves and refill the non-lighted sheath tank with PBS and reinstall it.

14 Empty the non-lighted waste tank in the sink while letting the water run and rinse the waste tank and sink, then reinstall waste tank.

15 Open the sort chamber and clean all surfaces including the hood with DI-H_2O and 70% ethanol, except the waste catcher. Be careful when wiping to not create static electricity.

16 Remove the 15 ml tube with 15% bleach from position 1.

17 When the shutdown procedure is finished, shut down PC and then the Bigfoot on the button on the right side of the instrument.